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Applicant: Dahm *et al.*

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Kathy Holloway

PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of the above-captioned application, please amend the application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows:

at page 6, line 1, replace "pad" with —pulp—;

at page 26, line 1, replace "pad" with —pulp—; and

at page 42, line 19, replace "step" with —stem—.

IN THE CLAIMS:

Please add claims 52-68 as follows:

—52. The method of Claim 4, wherein purification is effected by ion exchange chromatography.—

—53. The method of Claim 52, wherein ion exchange resin is a silica gel.—

—54. The method of Claim 7, wherein three standard nucleic acids are coamplified and are added in different concentrations to the sample.—

—55. The method of Claim 8, wherein:

the amplification product is quantified via a label; and

the label is selected from a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.—

—56. The method of Claim 9, wherein the label is a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.—

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—57. The method of Claim 11, wherein, as a positive control in the sample, a nucleic acid that occurs in peripheral blood is specifically amplified and detected.—

—58. The method of Claim 57, wherein the nucleic acid is mRNA that encodes a protein selected from among β-globin, glyceraldehyde-phosphate dehydrogenase, β-actin or a T-cell receptor.—

—59. The method of Claim 3, wherein as a negative control no reverse transcription reaction is carried out before the amplification reaction with the sample to be investigated and/or water is employed in place of the body fluid.—

—60. The method of Claim 16, wherein depletion is effected by immunoabsorption.—

—61. The method of Claim 17, wherein concentration is effected by immunoabsorption.—

—62. The method of Claim 20, wherein the cell separation medium has a density in the range of from 1.055 to < 1.070 g/ml.—

—63. The method of Claim 62, wherein the density is about 1.065 g/ml.—

—64. The method of Claim 25, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1.—

—65. The method of Claim 31, wherein at least one of the porous barrier, the filter or the sieve has a thickness of about 5 mm.—

—66. The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a pore size of 20-30 μm.—

—67. The method of Claim 1, wherein the tumor cells are derived from micrometastases of malignant tumors.—

—68. The kit of Claim 44, wherein the labeled oligonucleotide comprises the sequence 5' CGTTCTGGCT CCCACGACGT AGTC 3' SEQ ID No. 9, designated hTRTo or the corresponding reverse complementary sequence thereof.—

Please amend claims 1-51 as follows:

1. (Amended) [Method] A method for the quantification of tumor cells in a body fluid, [characterized in that] comprising:

(a) concentrating [the sample to be investigated is subjected to a method for concentrating] or depleting tumor cells in a sample of a body fluid; and

(b) specifically amplifying[a reaction is carried out, on the concentrated or depleted tumor cells, in which the] mRNA coding for the catalytic subunit of telomerase;[is specifically amplified,] and

(c) quantitatively determining the amount of amplified nucleic acid[is determined quantitatively], thereby quantifying tumor cells in a body fluid.

2. (Amended) The method of [Method according to] Claim 1, further comprising[characterized in that] prior to amplification, preparing cDNA from [reverse transcription reaction in which] the mRNA contained in the sample [is transcribed into cDNA is carried out before the amplification reaction with the sample to be investigated].

3. (Amended) [Method according to Claims 1 or 2, characterized in that] The method of Claim 2, wherein, prior to preparing cDNA, the [a DNase reaction is carried out with] the sample is treated with a DNAase[to be investigated before the transcription of the mRNA into cDNA].

4. (Amended) [Method according to any of Claims 1 - 3, characterized in that] The method of Claim 1, wherein the sample [to be investigated] is purified[, preferably by an ion exchange chromatography, in particular on silica gel].

5. (Amended) [Method according to any of Claims 1 - 4, characterized in that] The method of Claim 1, wherein[,] for quantitative determination of the telomerase-coding nucleic acid, the amplification products are labeled [even] during amplification and the amplification kinetics are measured continuously, including [even] during the amplification process.

6. (Amended) [Method according to] The method of Claim 5, [characterized in that] wherein a probe [which] that is specific for the amplification products, and [which] that emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification.

7. (Amended) [Method according to any of Claims 1 - 4, characterized in that] The method of Claim 1, wherein for quantitative determination of the telomerase-encoding nucleic acid, at least one[, preferably three,] standard nucleic [acids are] acid molecule is coamplified and [are] added in different concentrations to the sample[to be investigated].

8. (Amended) [Method according to any of Claims 1 - 7, characterized in] The method of Claim 1, wherein [that] the amplification product is quantified either directly or via a label[, preferably via a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label].

9. (Amended) [Method according to any of Claims 1 - 7, characterized in that] The method of claim 1, wherein the amplification product is detected via [a] hybridization with a labeled oligonucleotide[, where the label is preferably a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label].

10. (Amended) [Method according to any of Claims 7 - 9, characterized in that] The method of Claim 7, wherein quantification of[, to quantify] the telomerase-encoding nucleic acid is effected by comparing [to be determined,] the amount of coamplified nucleic acid or nucleic acids [is compared] with the amount of telomerase-encoding nucleic acid.

11. (Amended) [Method according to any of Claims 1 - 10, characterized in that] The method of claim 1, wherein the sample [to be investigated] is peripheral blood[, and in that a reaction is carried out with the sample to be investigated as positive control, in which a nucleic acid which occurs in peripheral blood, preferably the mRNA coding for β-globin, glyceraldehyde-phosphate dehydrogenase, β-actin or the T-cell receptor, is specifically amplified and detected].

Q2 cont

12. (Amended) [Method according to Claim 1 or any of Claims 3 - 11, characterized in that] The method of Claim 1, wherein as a negative [controls] control[, no reverse transcription reaction is carried out before the amplification reaction with the sample to be investigated and/or] water is employed in place of the body fluid.

13. (Amended) [Method according to any of Claims 1 - 12, characterized in that] The method of Claim 1, wherein one or both of the following oligonucleotide primers are used for the amplification:
5′ CTACCGGAAG AGTGTCTGGA GCAAGTTGGA AAGC 3′ SEQ ID No. 1, designated [(]hTRT1[)];
[and/or] and
5′ GGCATACCGA CGCACGCAGT ACGTGTTCTG 3′ SEQ ID No.2, designated [(]hTRT2[)],
wherein each of [where] hTRT1 [and/or] and hTRT2 optionally further comprises [where appropriate] a promoter sequence for an RNA polymerase.

14. (Amended) [Method according to any of Claims 1 - 13, characterized in that] The method of Claim 1, wherein amplification is effected with a DNA polymerase or an RNA polymerase[is used for the amplification].

15. (Amended) [Method according to any of Claims 1 - 14, characterized in that] The method of claim 14, wherein[, in the case of] if amplification is effected with a DNA polymerase, the amplification reaction is a [the] polymerase chain reaction (PCR)[is carried out] and, [in the case of] if

amplification is effected with an RNA polymerase, the reaction is an isothermal nucleic acid sequence-based amplification (NASBA) reaction[is carried out].

16. (Amended) [Method according to any of Claims 1 - 15, characterized in that] The method of claim 1, wherein the sample [to be investigated] is blood that is[, and in that the blood sample to be investigated is] depleted in stem cells and/or activated immune cells[, preferably by immunoabsorption].

17. (Amended) [Method according to any of Claims 1 - 16, characterized in that] The method of Claim 1, wherein the sample [to be investigated] is blood, and the tumor cells from the blood sample[to be investigated] are concentrated[, preferably by immunoabsorption].

18. (Amended) [Method according to any of Claims 1 - 17, characterized in that] The method of Claim 1, wherein the cells contained in the sample are cultivated under conditions [which] that are unfavorable for telomerase-positive nontumor cells but favorable for the tumor cells present.

19. (Amended) [Method according to Claim 18, characterized in that] The method of Claim 18, wherein the duration of the cultivation is such that nontumor cells die and tumor cells survive.

20. (Amended) [Method according to any of Claims 1 - 19, where,] The method of Claim 1, wherein for concentrating the tumor cells, a cell separation medium is covered with a layer of the body fluid and centrifuged[, characterized in that the cell separation medium has a density in the range from 1.055 to < 1.070 g/ml].

21. (Amended) [Method according to Claim 20, characterized in that] The method of Claim 20, wherein the cell separation medium has a density in the range of from 1.060-1.067 g/ml[and preferably of about 1.065 g/ml].

22. (Amended) [Method according to Claim 20 or 21, characterized in that] The method of Claim 20, wherein the centrifugation is carried out at about 1000 x g for about 30 minutes.

23. (Amended) [Method according to any of Claims 20 - 22, characterized in that] The method of Claim 20, wherein the cell separation medium used is Percoll or Ficoll.

24. (Amended) [Method according to any of Claims 20 - 23, characterized in that the body fluid is,] The method of Claim 20, wherein prior to applying the body fluid sample to the cell separation medium, it is mixed [being applied as a covering layer, admixed] with one or more substances [which] that prevent aggregation of platelets to tumor cells, and/or [the body fluid is,] prior to [being applied as a covering layer,] applying the body fluid sample to the cell separation medium, it is freed of substances [which] that promote aggregation of platelets to tumor cells.

25. (Amended) [Method according to any of Claims 20 - 24, characterized in that] The method of Claim 20, wherein the body fluid is peripheral blood.

26. (Amended) [Method according to Claim 25, characterized in that] The method of Claim 25, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent[, preferably in a ratio of about 1:1].

27. (Amended) [Method according to Claim 25 or 26, characterized in that] The method of Claim 25, wherein the peripheral blood is venous or arterial blood.

28. (Amended) [Method according to any of Claims 20 - 24, characterized in that] The method of Claim 20, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

29. (Amended) [Method according to any of Claims 20 - 28, characterized in that] The method of Claim 20, wherein [the centrifugation vessel is,] after centrifugation and before the tumor-cell-enriched interphase,

the centrifugation vessel is removed[,] and cooled intensively to prevent mixing of the cells in the different layers.

Q2 cont

30. (Amended) [Method according to any of Claims 20 - 29, characterized in that] The method of Claim 20, wherein the centrifugation is carried out in a vessel [which] that is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment and the body fluid is introduced into the upper compartment.

31. (Amended) [Method according to] The method of Claim 30, wherein [characterized in that] at least one of the porous barrier, the filter or the sieve [have] has a thickness of 1-10 mm[, preferably about 5 mm].

32. (Amended) [Method according to Claim 30 or 31, characterized in that] The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve [have] has a pore size of 20-100 μm[, preferably 20-30 μm].

33. (Amended) [Method according to any of Claims 30 - 32, characterized in that] The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve [are] is fabricated from [made of] a hydrophobic material or coated with a hydrophobic material.

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34. (Amended) [Method according to any of Claims 20 - 33, characterized in that] The method of Claim 20, wherein the cell separation medium contains a dye, [which makes the] whereby the color of the cell separation medium is distinguishable from that of the supernatant body fluid[, thus simplifying the localization of the interphase].

35. (Amended) [Method according to any of Claims 1 - 34, characterized in that] The method of Claim 1, wherein: the sample [to be investigated] is blood;

the method is performed[and in that there is an investigation in said method of], on [the one hand,]a venous blood sample and on [he other hand,] an arterial blood sample;[,] and the results from each are compared with one another.

36. (Amended) [Method according to any of Claims 1 - 35, characterized in that] The method of Claim 1, wherein: the sample [to be investigated] is blood;

the method is performed[, and in that there is an investigation in said method of,] on [the one hand,] a blood sample from the finger pad and, on [the other hand,] a venous or arterial blood sample;[,] and

the results from each are compared with one another.

37. (Amended) [Method according to any of claims 1 - 36, characterized in that] The method of Claim 1, wherein the tumor cells are derived from metastases[, preferably micrometastases,] of malignant tumors.^C

38. (Amended) [Method according to any of Claims 1 - 37, characterized in that] The method of Claim 1, wherein the tumor cells are selected from [a group of] cells of metastasizing tumors and/or neoplasms, wherein the cells [which] are derived from tumors and cells selected from the group consisting of a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma [and/or] and lymphoma cells.

39. (Amended) An oligonucleotide [Oligonucleotide] primer, [with] comprising the sequence

5′ CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3′ SEQ ID No. 1, designated [(]hTRT1[) and/or] or

5′ GGCATACCGA CGCACGCAGT ACGTGTTCTG 3′ SEQ ID No. 2, designated [(]hTRT2[)],

[where] wherein hTRT1 [and/or] and hTRT2 [may, if appropriate, additionally] optionally comprise a promoter sequence for an RNA polymerase.

40. (Amended) An oligonucleotide [Oligonucleotide] probe[with], comprising the sequence

5' CGTTCTGGCT CCCACGACGT AGTC 3'SEQ ID No. 9, designated [(]hTRT o[)] [and/or] or the corresponding reverse complementary sequence thereof.

41. (Amended) A kit [Kit] for the quantification of tumor cells in a body fluid, comprising[: (a)] an oligonucleotide primer pair for specific amplification of mRNA encoding the catalytic subunit of telomerase [telomerase-encoding mRNA].

42. (Amended) The kit of [Kit according to] Claim 41, [characterized in that] wherein the oligonucleotide primer pair specified [in (a) has the following sequences] comprises one or both of :

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' SEQ ID No. 1, designated [(]hTRT1[)]

[and/or] and

5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' SEQ ID No. 2, designated [(]hTRT2[)],

[where] wherein hTRT1 [and/or] and hTRT2 [comprises where appropriate] optionally further comprise a promoter sequence for an RNA polymerase.

43. (Amended) The kit of [Kit according to either of Claims 41 or 42, characterized in that it additionally comprises (b)] Claim 41, further comprising a standard nucleic acid or standard nucleic acids for coamplification.

44. (Amended) The kit of Claim 4, [Kit according to any of Claims 41 - 43, characterized in that it additionally comprises] further comprising a labeled oligonucleotide for detecting the amplified nucleic acid of the sample to be determined and/or one or more labeled oligonucleotides for detecting the coamplified standard nucleic acid or standard nucleic acids[, in particular an oligonucleotide with the sequence:

5' CGTTCTGGCT CCCACGACGT AGTC 3'(hTRTo)

and/or the corresponding reverse complementary sequence thereof].

45. (Amended) [Kit according to any of Claims 41- 44, characterized in that it additionally comprises] A kit of Claim 41, further comprising one or more of a reverse transcriptase, a DNA polymerase[, preferably a Taq polymerase,] a DNase, [and/or suitable] buffers[and, where appropriate], labeled nucleotides[and, where appropriate], means [suitable] for the depletion of stem cells and/or activated immune cells [and/or] and means for the concentration of tumor cells.

46. (Amended) [Kit according to any of Claims 41 - 45, characterized in that it additionally comprises] A kit of Claim 41, further comprising one or more of a reverse transcriptase, an RNA polymerase[, preferably a T7 RNA polymerase,] an RNase H, a DNase[and/or suitable] buffers[and, where appropriate], labeled nucleotides and[, where appropriate], means [suitable] for the depletion of stem cells and/or activated immune cells and/or for the concentration of tumor cells.

47. (Amended) [Kit according to any of Claims 41 - 46, characterized in that it additionally comprises] The kit of Claim 41, further comprising a cell separation medium having a density in the range of from 1.055 to < 1.070 g/ml and[, if appropriate,] optionally a centrifugation vessel.

48. (Amended) [Kit according to] The kit of Claim 47, [characterized in that] wherein the cell separation medium has a density in the range of from 1.060 to 1.067 g/ml[and preferably of about 1.065 g/ml].

49. (Amended) [Kit according to either of Claims 47 or 48, characterized in that] The kit of Claim 47, wherein the centrifugation vessel has a porous barrier, a filter or a sieve of a thickness of 1-10 mm[, preferably of about 5 mm], which [divide] divides the centrifugation vessel into an upper and a lower compartment.

50. (Amended) [Kit according to] The kit of Claim 49[, characterized in that] wherein the porous barrier, the filter or the sieve [have] has a pore size of 20-100 μm[, preferable 20-30 μm].

51. (Amended) [Kit according to Claim 49 or 50, characterized in that] The kit of Claim 49, wherein the cell separation medium is in the lower compartment of the centrifugation vessel.

REMARKS

Claims 1-68 are presently pending. The claims are amended and new claims added herein to delete multiple dependencies and to put the claims into a form that better comports with U.S. practice. Therefore, no new matter has been added.

It is respectfully requested that any references of record in the International stage of prosecution of this application be made of record in this application.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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